REMARKS

The July 8, 2002 Official Action and references cited therein have been carefully reviewed. In light of the amendments presented herewith and the following remarks, favorable reconsideration and allowance of the application are respectfully requested.

In accordance with the present amendment, the specification has been amended at page 6 and 22 to clarify certain experimental results that are set forth in a latter part of the specification. Support for these amendments can be found at page 6, lines 15-32 and page 22, line 14 over to page 23, line 16. Additionally, Sequence Identifiers have been inserted at pages 5, 6, and 22. These amendments do not constitute new matter.

The Examiner has indicated that there are certain minor informalities in the application which require correction. Specifically, the Examiner notes that the application does not contain an abstract, and that the title is not descriptive. To rectify these informalities, an abstract of the disclosure is submitted herewith. Also the title has been amended to render it more descriptive of the invention presently claimed.

At page 2 of the Official Action, the Examiner has objected to claim 19 being in improper form indicating that a multiple dependent claim should refer to the base claims in the alternative only.

The Examiner has rejected Claims 21, 22, 31, and 40 under 35 U.S.C. §101, as allegedly being drawn to non-statutory subject matter.

At page 3 of the Official Action, the Examiner has rejected Claims 1-40 under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one of skill in the art that the inventors had possession of the claimed invention at the time the application was filed.

The Examiner has further rejected Claims 1-40 under 35

U.S.C. §112, first paragraph, asserting that the specification allegedly fails to provide enablement commensurate in scope with the present claims.

At page 7 of the Official Action, the Examiner has further rejected Claims 17, 30, and 39 under 35 U.S.C. §112, first paragraph, asserting that the specification allegedly fails to provide adequate enablement for practicing the invention as claimed.

The Examiner has rejected Claims 7, 12-27, 31, and 40 under 35 U.S.C. §112, second paragraph, as allegedly indefinite for failing to particularly point out and distinctly claim the subject matter regarded as the invention.

Claims 1-11 and 20-22 stand rejected under 35 U.S.C. §103 (a) as allegedly unpatentable over van Engelen et al., (Transgenic Research 4, pp.288-290, 1990) in view of Canton et al., (Plant Molecular Biology 22, pp. 819-828, 1993). Further, at page 12 of the Official Action, the Examiner has rejected Claims 12-40 under 35 U.S.C. §103 (a) as allegedly unpatentable over Donn et al., (EP0303780 1989-02-22), in view of Feuiller et al., (Plant Molecular Biology 27, pp. 651-667, 1995).

The foregoing constitutes the entirety of the objections and rejections raised in the July 8, 2002 Official Action. In light of the present claim amendments and the following remarks, each of the above-noted rejections under 35 U.S.C. § 101, 112, first and second paragraphs, and 103 is respectfully traversed.

CLAIM 19 AS AMENDED IS A PROPER MULTIPLE DEPENDENT CLAIM

The Examiner has objected to claim 19 being in improper form for not referring to base claims in the alternative. The claim has been amended to remove the multiple dependency and now explicitly incorporates the features from claim 9, thereby rendering this objection moot.

CLAIMS 21, 22, 31, AND 40 AS AMENDED FULLY MEET THE REQUIREMENTS OF 35 U.S.C. 101

The Examiner has rejected Claims 21, 22, 31, and 40 under 35 U.S.C. §101, as being drawn to non-statutory subject matter. The Examiner states that a reproductive unit and/or a cell from a transgenic plant may include plant matter which does not contain tDNA, and thus reads on a product of nature.

The claims as amended require that the cell or reproductive unit contain the plant expression cassette or transgene of claim 2, and thus cannot be considered a product of nature. Accordingly, Applicants request that the rejection of claims 21, 22, 31, and 40 on this basis be withdrawn.

CLAIMS 1-40 AS AMENDED ARE FULLY DESCRIBED BY THE DISCLOSURE IN THE SPECIFICATION

The Examiner has rejected Claims 1-40 under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter not described in the specification in such a way as to reasonably convey to one of skill in the art that the inventors had possession of the claimed invention at the time the application was filed.

The Examiner alleges that the disclosure only describes the glutamine synthetase coding sequence disclosed in Canton et al. Plant Molecular Biology, 22 (5), 819-828, 1993, and that the instant disclosure fails to describe the composition or structure of other glutamine synthetase cDNAs or any nucleic acids having 70% sequence identity to any cDNAs. The Examiner cites *University of California V. Eli Lilly and Co.*, 43 USPQ2d 1398 (Fed. Cir. 1997) as support for this contention.

Applicants respectfully traverse this rejection as it applies to the newly amended claims. In *University of California V. Eli Lilly and Co.*, the courts ruled that disclosure of a process for obtaining cDNA from a particular organism and description of the encoded protein failed to

provide written description of the actual cDNA from the organism which would encode the disclosed protein, despite the disclosure of a cDNA encoding that protein from another organism. The court also addressed the manner by which a genus of cDNAs might be described:"A description of a genus of cDNAs may be achieved by means of a recitation of a representative number of cDNAs, defined by nucleotide sequence, falling within the scope of the genus or of a recitation of structural features common to the members of the genus, which features constitute a substantial portion of the genus."

Further, As noted in the MPEP at § 2163,

> To satisfy the written description requirement, a patent specification must describe the claimed invention in sufficient detail that one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention.

Furthermore, the written description guidelines set forth in the Federal Register Vol. 66, No. 4, January 5, 2001 states that "An adequate written description of the invention may be shown by any description of sufficient, relevant, identifying characteristics, so long as a person skilled in the art would recognize that the inventor had possession of the claimed invention."(page 1105, column 3). "An applicant may also show that an invention is complete by disclosure of sufficiently detailed, relevant identifying characteristics which provide evidence that the applicant was in possession of the claimed invention, ie: complete or partial structure, other physical and/or chemical properties, functional characteristics when coupled with a known or disclosed correlation between function and structure, or some combination of characteristics." (Page 1106, column 1). Specifically with regard to genus claims, the guidelines state that (2) For each claim drawn to a genus: The written description requirement for a claimed genus may be satisfied through a sufficient description of a representative number of species by actual reduction to practice...reduction to drawings...or by disclosure of relevant identifying

characteristics, i.e. structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show applicant was in possession of the claimed genus..." (Page 1106, column 3)

Applicants submit that the instant claims clearly meet this requirement. First, the structure of glutamine synthetase is clearly described. Genbank Accession No. X69822 is explicitly disclosed in the instant application, and provides the specific structure of the claimed genus in question. Further, the function of glutamine synthetase is known, and has been correlated to a specific activity (relevant identifying characteristics) in the instant claims, which now recite that the transfection of plants with a construct for over expressing glutamine synthetase results in increased growth rate and productivity. Thus the claims meet the requirement that a structure is described, and that the described structure is correlated to a function.

Therefore, in light of the foregoing claim amendments and remarks, Applicants respectfully submit that the claims are amended comply with all the requirements of 35 U.S.C. §112, first paragraph and request that the rejection of amended claims 1-40 under 35 U.S.C. §112, first paragraph be withdrawn.

CLAIMS 1-40 AS AMENDED ARE FULLY ENABLED BY THE DISCLOSURE IN THE SPECIFICATION

The Examiner has rejected Claims 1-40 under 35 U.S.C. §112, first paragraph, asserting that the specification allegedly fails to provide enablement commensurate in scope with the present claims.

The Examiner sets forth that the specification does not enable the full scope of claims for the following reasons.

(1) The Examiner alleges that the instant specification only provides one glutamine synthetase coding sequence.
(2) The Examiner next argues that the methods are only tested in the INRA 7171-B4 plant clone.
(3) The Examiner then asserts that there is no evidence that the instantly claimed transgenic plants exhibit any other improved growth except improved height.
(4) The Examiner then contends that conserved regions of a sequence must be known in order to support any variation of a sequence if there is limited sequence information available, citing a paper which discusses NBS disease resistance.
(5) The Examiner further alleges that determination of various hybridization and PCR conditions and reagents would require undue experimentation.
(6) The Examiner last argues that transformation of plants in the genus Populus is complex and also requires undue experimentation.

A rejection under 35 U.S.C. §112, first paragraph based on inadequate enablement is proper only when the rejected claims are of such breadth as to read on subject matter as to which the specification is not enabling. In re Borkowski 164 U.S.P.Q. 642 (CCPA 1970).

With regard to the argument that (1) the instant specification only provides one glutamine synthetase coding sequence, while there may only be one exemplified sequence, it would require only routine experimentation to screen for and isolate, or determine other glutamine synthetase molecules. The instant specification discloses Genbank Accession No. X69822 which encodes glutamine synthetase from Pinus sylvestris. A search of the GenBank database using the search term "glutamine synthetase" reveals over 100 glutamine synthetase encoding nucleic acids. Applicants submit that the skilled person is aware of the sequence data present in the GenBank database Thus, determination of other glutamine synthetase sequences would require only routine

experimentation, since the sequence and enzymatic function of the instant glutamine synthetase is known and disclosed herein, and because methods of conservative amino acid substitution, computer modeling, sequence data base searching and screening techniques allow the skilled artisan to rapidly, efficiently, and routinely screen polynucleotides and polypeptides for variants, orthologs and homologs of a known sequence. Therefore it would require mere routine experimentation to screen for variants of the instant glutamine synthetase coding sequence, or others which have the same activity.

With regard to the argument that (2) the methods are only tested in the INRA 7171-B4 plant clone, the clone exemplified is an art recognized model for plant cloning and thus is considered representative of the genus. Further, the successful transformation of a species is strong evidence of an expectation of success in the absence of evidence to the contrary. In this case, the Examiner has provided no evidence or sound scientific reasoning as to why the exemplified clone would not be representative of a plant genus. The Examiner is respectfully pointed to MPEP 2164.02, which addresses working examples, stating that "For a claimed genus, representative examples, together with a statement applicable to the genus as a whole will ordinarily be sufficient [for enablement] if one skilled in the art...would expect that the claimed genus could be used in that manner without undue experimentation." In the instant case, the clone used is a plant system model and thus would be expected to be representative of the genus of plants.

With regard to the argument that (3) there is no evidence that the instantly claimed transgenic plants exhibit any other improved growth except improved height, the Examiner is respectfully pointed to Example 2, which described the phenotypic characteristics of transformed plants. At pages 34-37, for example, the specification sets forth experimental data revealing 1) the increased chlorophyll concentration, 2)

the increased soluble protein content, 3) the increased height, 4) the increased leaf length, 5) the increased leaf width, 6) increased leaf area, 7) increased leaf number, and 8) increased photosynthetic area in transformed plants over control. It is respectfully submitted that this combination of phenotypic alterations reflect an increase in "growth" of the plant.

With regard to the argument that (4) that conserved regions of a sequence must be known in order to support any variation of a sequence if there is limited sequence information available, citing a paper which discusses NBS disease resistance, it is first noted that the novelty of the instant invention lies in the unexpected superior properties of Poplar plants expressing exogenous glutamine synthetase. The paper cited by the Examiner discusses plant disease resistance genes and is in no way relevant to the instant application, which is drawn to transformation with a glutamine synthetase coding sequence. Genetic diversity in a completely different gene set would not necessarily correspond to the diversity of the instantly claimed glutamine synthetase. Additionally, the Examiner's comment that isolating homologous DNA sequences is problematic when a dearth of sequence information available is inapplicable to the instant invention, as no "dearth of sequence information" exists in the instant case. As noted in Canton et al., cited by the Examiner in the instant Office Action, "Recent molecular studies have shown that GS isoforms consist of polypeptides different in size which are encoded by nuclear encoded genes. In the past few years, a number of cDNAs have been isolated and characterized..."

With regard to the argument that (5) that determination of various hybridization and PCR conditions and reagents would require undue experimentation, guidance for the same is explicitly discussed in the specification, for example at page 11, line 30-page 13, line 8, and further, these conditions and

procedures are very well known in the art and would require only routine experimentation. For further guidance, please see Sambrook et al., Molecular Cloning, 1989, incorporated by reference at page 11 of the specification, which is a well established guide for such experimentation, and which would be recognized by the skilled artisan as such.

With regard to the argument that (6) that transformation of plants in the genus Populus is complex and would require undue experimentation, it is noted that applicant successfully transformed the genus Populus, in fact with enhanced transformation efficacy (see for example page 14, line 25-28, and Example 1). Thus clearly this particular construct exhibits increased transformation efficacy. The Examiner relies on Han et al. for his contention that transformation of Populus plants is unpredictable. It is respectfully noted that Han et al. is drawn to the transformation of two species of cottonwoods, and is not representative of Populus plants in general. It is further noted that the teachings of Han (published prior to the instant filing date) demonstrate means of transforming these recalcitrant species. It is also noted that numerous poplars have been transformed in laboratories (page 464). Finally, in the instant case, Populus transformation was conducted quite successfully, with a transformation rate of nearly 100% (see page 14 of the instant specification.)

In light of the foregoing claim amendments and remarks, Applicants respectfully submit that the claims as amended comply with all the requirements of 35 U.S.C. §112, first paragraph and request that the rejection of amended claims 1-40 under 35 U.S.C. §112, first paragraph be withdrawn.

CLONE INRA717 1B4 IS WIDELY USED IN THE ART

The Examiner has rejected Claims 17, 30, and 39 under 35 U.S.C. §112, first paragraph, asserting that the specification

allegedly fails to provide enablement for the invention, because the claimed plants required to practice the invention have not been deposited.

The Examiner states that the hybrid Poplar (*Populus tremula x P. alba*) clone INRA 717 1-B4 is required to practice the claimed invention. Applicants take exception to the Examiners position in this regard. The Examiner asserts that without a publically available deposit of the above, one of ordinary skill in the art could not be assured of the ability to make the hybrid poplar clone in the same manner as claimed. The Examiner thus concludes that given the lack of guidance in the specification, and the inability of those in the art to reproduce clones of the hybrid poplar clone, it would require undue experimentation for one of skill in the art to identify and obtain the starting material for transformation.

Applicants respectfully traverse the Examiner's contention, and set forth the following argument and evidence that the invention may be practiced without undue experimentation, without depositing the hybrid Poplar (*Populus tremula x P. alba*) clone INRA 717 1-B4.

Applicants respectfully submit that the hybrid Poplar (*Populus tremula x P. alba*) clone INRA 717 1-B4 is readily known and available to the public. See MPEP 2404.01.

In the instant case, the hybrid Poplar (*Populus tremula x P. alba*) clone INRA 717 1-B4 is well known in the art. As set forth in the specification at page 25, lines 9-14, the maintenance of the clone is described in Leple et al., 1992, Plant Cell Reports 11: 137-141. Further, the INRA 717 1-B4 clone is widely used and available to the public, as evidenced by it's citation in numerous publications. Enclosed are the following documents which describe use of the INRA 717 1-B4 clone in experiments, all authored by different groups, which further support the clone's public availability:

Pilate, G. et al., Nature Biotechnology, Volume 20, pages 607-612, June 2002. The INRA 717 1-B4 clone is described at

page 611.

Han et al., Plant Cell Reports, Volume 19, pages 315-320, 2000. The INRA 717 1-B4 clone is described at page 318.

Tian et al., Tree Physiology, Volume 19, pages 514-546, 1999. The INRA 717 1-B4 clone is described at page 542.

Rennenberg et al., Sulfur Nutrition and Sulfur Assimilation in Higher Plants, pages 393-398, 2000. The INRA 717 1-B4 clone is described at page 393.

Accordingly, as it is readily apparent that this clone is available to the skilled person, the invention may be practiced without undue experimentation or deposit of this particular clone. Applicants respectfully submit that the claims comply with all the requirements of 35 U.S.C. §112, first paragraph and request that the rejection of claims 17, 30, and 39 under 35 U.S.C. §112, first paragraph be withdrawn.

CLAIMS 1-17 AS AMENDED FULLY COMPLY WITH THE DEFINITENESS REQUIREMENTS OF 35 U.S.C. §112, SECOND PARAGRAPH

At page 9 of the Official Action, the Examiner has rejected Claims 7, 12-27, 31, and 40 under 35 U.S.C. §112, second paragraph, as allegedly indefinite for failing to particularly point out and distinctly claim the subject matter regarded as the invention.

The relevant inquiry in determining whether a given claim satisfies the requirements of 35 U.S.C. §112, second paragraph, is whether the claim sets out and circumscribes a particular area with a reasonable degree of precision and particularity such that the metes and bounds of the claimed invention are reasonably clear. In re Moore, 169 U.S.P.Q. 236 (C.C.P.A. 1971). Applicants respectfully submit that with respect to Claims 12-27, 31, and 40 as amended, this inquiry must be answered in the affirmative.

The Examiner rejects claim 4 for reciting the limitation

"the glutamate coding sequence" in line 2, which the Examiner states lacks antecedent basis. Claim 4 has been canceled and thus this ground of rejection is no longer appropriate.

The Examiner rejects claim 7 for containing improper English. Additionally, the Examiner states that it is unclear if the nucleic acid sequence is in addition to the coding sequence of claim 1. Claim 7 has been amended to correct the minor grammatical error as well as to clarify that the claim encompasses glutamine synthetase encoding nucleic acids. The hybridization conditions which correspond to moderate stringency have also been recited in the claim. Support for this amendment can be found at page 12, lines 35-38. The Examiner asserts that the term identical in claim 7 is unclear. Applicants respectfully submit that one of ordinary skill in the art is fully cognizant of the meaning of this phrase in reference to a nucleic acid sequence. Additionally, the Examiner's attention is drawn to page 9, lines 11-30 where the meaning the terms are explicitly set forth. Claim 7 also requires that the variant glutamate synthetase sequences possess enzymatic function. The Examiner also contends that the reference to the GenBank accession number is unclear. Applicants submit that this recitation is in no way indefinite. The GenBank database clearly indicates that the sequence is from the Canton reference of 1993.

Claim 12 has been rejected because the recitation "transforming *in vitro*" is allegedly unclear. This grounds of rejection is not understood. The Examiner has not explained why the recitation "transforming *in vitro*" is unclear, and it is applicant's position that such a step is well known in the art to mean a transformation step (for example integrating a polynucleotide construct into a cell) which is conducted *in vitro* (for example, in an isolated cell or group of cells which is grown in culture). For an example of in vitro plant transformation, the Examiner attention is directed to pages 26-27 of the specification.

Claims 12 and 23 have been rejected for reciting the allegedly indefinite term "improved". It is applicant's position that one of skill in the art would recognize that "improved nitrogen metabolism" refers to an increase in the rate, productivity, etc. of any stage of nitrogen assimilation or consumption over a non-transformed plant. Without acquiescing to the Examiner's rejection, and solely to expedite prosecution, applicant has amended the claim to clarify that the nitrogen metabolism is improved over equivalent non-transformed plants. With regard to the Examiner's statement that the term nitrogen metabolism could refer to many different aspects of nitrogen metabolism, it is respectfully submitted that the term refers to any aspect of nitrogen metabolism. The Examiner is pointed to MPEP 2173.04, entitled Breadth is Not Indefiniteness.

> "Breadth is not to be equated with indefiniteness. In re Miller, 441 F.2d 689, 169 USPQ 597 (CCPA 1971). If the scope of the subject matter embraced by the claims is clear, and if applicants have not otherwise indicated that they intend the invention to be of scope different from that defined in the claims, the claims comply with 35 U.S.C. 112 second paragraph."

Applicants submit that in this case nitrogen metabolism may be broad, but the metes and bounds are clear: it pertains to any aspect of nitrogen metabolism.

Claim 12 has been rejected as allegedly lacking antecedent basis for the recitation of the term "said plant". It is respectfully submitted that "said plant" in line 2 of claim 12 refers to the recitation "a transformed plant" in line 1 of claim 12, and thus has antecedent basis.

Claims 18 and 19 have been rejected as improperly dependent. The Examiner appears to object to the language "wherein the transformation step uses...". It is applicant's position that this language is clear, because it clearly sets forth what step is being altered, and how it is being altered. However, in the interest of advancing prosecution, the Examiner's helpful suggestion has been adopted, and thus the rejection is believed overcome.

Claims 21, 31, and 40 are rejected for the allegedly indefinite recitation of "a reproductive unit". It is the Examiner's position that it is not clear if "a reproductive unit refers to a seed, a flower, or a sexual gamete". Applicants respectfully submit that the recitation is clear. A reproductive unit can refer to any of the aforementioned units. A reproductive unit broadly encompasses any unit which facilitates reproduction. It is noted again, as set forth above, that breadth does not equate to indefiniteness.

Claim 25 has been rejected for allegedly using improper English in the recitation of "genes is". This grammatical error has been corrected and thus the rejection is believed overcome.

Claim 27 is rejected because the limitation "from the family Salicaceae" allegedly fails to further limit the claim. The amendment to claim 27, which corrects dependency is believed to overcome this grounds of rejection.

Claim 27 is rejected for allegedly lacking antecedent basis in the recitation of "the transgenic plant of claim 17" because claim 17 is directed to a method. The amendment to claim 27, which corrects dependency is believed to overcome this grounds of rejection.

In view of the claim amendments presented herewith, Applicants respectfully submit that one of skill in the art would be readily appraised of the metes and bounds of the claims. Accordingly, the rejection of Claims 12-27, 31, and 40 as amended under 35 U.S.C. §112, second paragraph, is no longer appropriate and should be withdrawn.

CLAIMS 1-11 AND 20-22 AS AMENDED ARE NOT UNPATENTABLE OVER VAN ENGELEN ET AL., IN VIEW OF CANTON ET AL.

The Examiner has rejected Claims 1-11 and 20-22 under 35 U.S.C. §103 (a) as allegedly unpatentable over van Engelen et al., (Transgenic Research 4, pp.288-290, 1990) in view of Canton et al., (Plant Molecular Biology 22, pp. 819-828,

1993).

The relevant inquiry in determining obviousness under 35 U.S.C. §103 based on the combined disclosure of references, is whether the references supply some teaching or suggestion to one of ordinary skill in the art to arrive at the invention as claimed. In re Dow Chemical Company, 5 U.S.P.Q.2d 1529 (Fed. Cir. 1988). Obviousness cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching or suggestion supporting the combination. In re Fine, 5 U.S.P.Q.2d 1596 (Fed. Cir. 1988). Moreover, the teaching or suggestion supporting the desirability or the combination must be found in the prior art, not in the applicant's disclosure. In re Fritch, 23 U.S.P.Q.2d 1780 (Fed. Cir. 1992). Under these standards, none of the cited references, considered singly or in combination, renders obvious the claimed invention.

Claims 1, 2 and 7 have been amended to call for an expression cassette which comprises a 5' cauliflower mosaic virus promoter sequence operably linked to a glutamine synthetase encoding nucleic acid which is in turn operably linked to a 3' Nos terminator sequence.

van Engelen et al. teaches a plant expression cassette for Agrobacterium mediated plant transformation. Canton et al. teaches the characterization of a cDNA clone encoding a cytosolic portion of glutamine synthetase. The Examiner states that one would be motivated to combine these references because the plant expression cassette of van Engelen "was generally useful for any heterologous gene and improved the efficiency of transformation using Agrobacterium."

The claims are drawn to an expression cassette, methods of use thereof and transgenic plant comprising the same which confer enhanced nitrogen assimilation and superior phenotypic characteristics upon transgenic plants expressing the same.

Neither van Engelen et al. nor Canton et al., alone or combined suggest transforming a plant with an expression

cassette comprising glutamine synthetase. The mere fact that such an expression cassette could be made is not motivation to make the cassette, nor is it motivation to transform a plant with the same. In fact, Canton et al. states that "little is known about GS in gymnosperms particularly at the molecular level." (Page 819, column 2). Thus it is clear that there is no motivation to practice the methods of the instant claims, based on the teachings of van Engelen et al. and Canton et al., and further neither reference teaches plant transformation which results in increased nitrogen assimilation or altered phenotype.

Further, the references fail to teach the unexpectedly increased transformation efficacy and plant growth which results from the instantly claimed methods. As set forth in the disclosure as filed, the instant expression cassettes exhibited surprisingly increased transformation efficacy (100% when the rate typical of woody plants is 15-20% - page 14, lines 25-28). Further, the transformed plants exhibited significant phenotypic changes, including increased height, leaf number, leaf width, and photosynthetic area, etc. (see Example 2, pages 32-37.) These results would have been unexpected, in light of the previous lack of success of others in using glutamine synthetase to increase growth (see page 3, lines 18-28 of the specification)

The Examiner is pointed to MPEP 716.02, which discusses unexpected results, which are evidence of non-obviousness.

In light of the foregoing, it is respectfully submitted that claims 1, 2, 7 and 20-22 are patentable over van Engelen et al. in view of Canton et al.

CLAIMS 12-40 AS AMENDED ARE NOT UNPATENTABLE OVER DONN ET AL. IN VIEW OF FEUILLER ET AL.

At page 12 of the Official Action, the Examiner has rejected Claims 12-40 under 35 U.S.C. §103 (a) as allegedly unpatentable over Donn et al., (EP0303780 1989-02-22), in view

of Feuiller et al., (Plant Molecular Biology 27, pp. 651-667, 1995).

The relevant inquiry in determining obviousness under 35 U.S.C. §103 is set forth above.

At the outset, it is noted that Donn et al. is an International Patent Publication which is written in German. Only the abstract of Donn et al. is in English. Although the Examiner has cited portions of Donn et al. which are written in German (all of Example 1), it is applicant's understanding that such a citation is improper. If the Examiner is relying on more than the abstract of Donn et al. to support this rejection, he is respectfully requested to provide applicant with a translation of the document so that applicant can address the reference. It is also noted that Canton at el is not cited in the Examiner's statement of rejection, yet it is included in the text of the rejection. While it is unclear to Applicant if the Examiner intended to cite Canton et al. in this rejection or not, Applicant will will assume that the rejection is based on the inclusion of Canton et al.

The abstract of Donn et al. states that "Plants which overproduce glutamine synthetase have improved utilization of nitrogen. They therefore thrive on soils which, because the supply of nitrogen which can be utilized by plants is too small, allow virtually no useful growth in corresponding wild types." The Examiner states that Donn et al. teaches glutamine synthetase overproduction in transgenic plants transformed with CAMV-alpha glutamine synthetase by Agrobacterium tumifaciens mediated transformation, but fails to teach transformation of hybrid poplar (Populus tremula x Popular alba) clone INRA 717 1-B4 with a pBIN19 vector comprising glutamine synthetase from Pinus sylvestris. The Examiner supplements this deficiency by citing Feuiller et al., which teaches the relevant hybrid poplar transformed with pBIN198 to express a different proteins. The Examiner then cites Canton et al. for it's teachings of the GS cDNA

sequence. The Examiner concludes that it would be obvious to transform poplar plants with glutamine synthetase because Donn et al. teach that glutamine synthetase over expression increases nitrogen efficiency and Feuiller et al. provides the means of transforming poplars, while Canton et al. supplies the cDNA of glutamine synthetase.

The claims are drawn to cassettes and methods of using the same to transform poplar plants to over express glutamine synthetase thereby producing a phenotypic alteration which includes increased growth.

As set forth above, while Donn et al. in general discusses that transformation of plants may increase nitrogen utilization, Donn et al. does not address transformation of poplars, which are difficult to transform, as set forth above. Donn et al. also does not address phenotypic change (increased growth), but instead discusses growth under nitrogen. starvation.

Further the references fail to teach the unexpectedly increased transformation efficacy and plant growth which results from practicing the instantly claimed invention. It is a well-settled premise in patent law that "silence in a reference is not a proper substitute for adequate disclosure of facts from which a conclusion of obviousness may justifiably follow". In re Burt, 148 U.S.P.Q. 548 (CCPA 1966) As set forth above, and in the disclosure as filed, the instant expression cassettes exhibited surprisingly increased transformation efficacy (100% when the rate typical of woody plants is 15-20% - page 14, lines 25-28). Further, the transformed plants exhibited significant phenotypic changes, including increased height, leaf number, leaf width, and photosynthetic area, etc. (see Example 2, pages 32-37.) Both of these results would have been unexpected, in light of the previous lack of success of others in using glutamine synthetase to increase growth (see page 3, lines 18-28 of the specification)

It is respectfully submitted that in the instant case, the introduction of the glutamine synthetase vector into Poplar results in an unexpectedly increased transformation efficacy, as well, as unexpectedly enhanced plant height, leaf areas, etc.

Accordingly, Applicants submit that the claims 12-40 are not unpatentable over Donn et al. in view of Feuiller et al. and respectfully request that the rejection of the claims under §103 be withdrawn.

CONCLUSION

In view of the amendments and remarks presented herein, it is respectfully urged that the rejections set forth in the July 8, 2002 Official Action be withdrawn and that this application be passed to issue. In the event the Examiner is not persuaded as to the allowability of any claim, and it appears that any outstanding issues may be resolved through a telephone interview, the Examiner is requested to telephone the undersigned attorney at the phone number given below.

Respectfully submitted,

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Enclosures: Appendix A

Appendix A

In the Specification:

At page 1 line 3 please replace the existing paragraph with the following paragraph: This application is a §371 Application of PCT/US99/18267, filed August 11, 1999 which in turn claims priority to US Provisional Application No. 60/096,032, filed August 11, 1998 the entire disclosure of which is incorporated by reference herein. [This application claims priority to US 60/096,032, filed August 11, 1998, the entire disclosure of which is incorporated by reference herein.]

Please replace the paragraphs at page 6, lines 3-32 of the specification with the following substitute paragraphs:

Another aspect of the invention is a transgenic woody perennial plant with improved nitrogen [metablism] metabolism which comprises at least one transgene expressing the coding sequence of glutamine synthetase. In preferred embodiments, the glutamine synthetase gene is from a gymnosperm, from *Pinus sylvestris*, and is Genbank Accession No. X69822. In other preferred embodiments, the transgenic plant is in the family Salicaceae, the genus Populus, is a hybrid *Populus tremula* X *P. alba*, and is clone INRA 717 1-B4 of the hybrid *Populus tremula* X *P. alba*. This aspect additionally includes a reproductive unit from the transgenic plant.

Another aspect of the invention is a transgenic woody perennial that exhibits a growth rate over the first [two] three months in the greenhouse that is at least [XX]10% greater than that of equivalent untransformed plants. In a preferred embodiment, the plant additionally exhibits a protein concentration (g/gfw) that is at least 10% greater than that of equivalent untransformed plants after the first

[XX] 3 months in the greenhouse. In a most preferred embodiment, the transgenic plant additionally exhibits a chlorophyll concentration (g/gfw) that is at least 10% greater than that of equivalent untransformed plants after the first [XX] 3 months in the greenhouse. In other preferred embodiments, the plant is in the family Salicaceae, in the genus Populus, a hybrid of *Populus tremula* X *P. alba*, and is clone INRA 717 1-B4 of the hybrid *Populus tremula* X *P. alba*. This aspect additionally contains a reproductive unit of the transgenic plant.

Please replace the paragraph which begins at page 22, line 14, and ends page 23, line 16 of the specification with the following substitute paragraph.

Also provided in accordance with the current invention is a poplar tree that has a statistically significant higher growth rate, higher protein and chlorophyll content in mature leaves, and larger mature leaf dimensions than its untransformed equivalent. In a preferred embodiment, this transgenic tree exhibits at least 10% greater growth rate during the first 3 months in the greenhouse after transformation as compared to untransformed trees of the same cultivar. More preferably, the transgenic poplar is 40% greater, and in a most preferred embodiment, the transgenic tree is 60% greater. In a more preferred embodiment, the transgenic poplar additionally has at least 10% greater grams of protein in the leaf tissue at [XX]3 months per gram fresh weight as compared to untransformed trees of the same cultivar. More preferably, the tree exhibits at least 15% greater protein, and most preferably, the tree exhibits at least 25% greater protein per gram per gram fresh weight. In a particularly preferred embodiment, the transgenic poplar additionally has at least 10% greater grams of chlorophyll per

gram fresh weight in mature leaf tissue as compared to control trees of the same cultivar. In a more preferred embodiment, the trees have at least 15% greater chlorophyll, and in a most preferred embodiment, the trees have at least 20% greater chlorophyll per gram per gram fresh weight. In a more particularly preferred embodiment, the transgenic poplar additionally has at least 10% greater area per mature leaf as compared to control trees of the same cultivar. In a more preferred embodiment, the trees have at least 15% greater leaf area, and in a most preferred embodiment, the trees have at least 20% greater leaf area per leaf. In regards to the present invention, statistical significance of quantified differences is determined using one-way analysis of variance (ANOVA). This statistical test is well known to those in the art, and computer programs that carry out this test are commercially available. The level of probably (P) used is 0.05 in a preferred embodiment, 0.01 in a more preferred embodiment, and 0.001 in a most preferred embodiment.

Claim amendments

1. (Amended) A plant expression cassette, which comprises a 5' cauliflower mosaic virus 35S promoter operably linked to a nucleic acid encoding a glutamine synthetase [gene coding sequence] protein [promoter] and a 3' NOS terminator sequence, wherein expression of said cassette in a plant increases nitrogen metabolism in said plant.

2. (Amended) The expression cassette of claim 1, wherein the glutamine synthetase coding sequence is from [a] gymnosperm *Pinus sylyvestris* having Genbank Accession No. X69822.

7. (Amended) The expression cassette of claim 1, [which

is contains a nucleic acid] wherein said glutamate synthetase sequence is selected from the group consisting of:

A.) a nucleic acid sequence that is at least 70% identical to Genbank Accession No. X69822 and encodes a protein having enzymatic function;

B.) a nucleic acid sequence that encodes a protein that is at least 70% similar to Genbank Accession No. X69822 and encodes a protein having enzymatic function;

C.) a nucleic acid sequence that hybridizes to Genbank Accession No. X69822 at moderate stringency with hybridization in 6X SSC, 5X Denhardt's solution, 0.5% SDS and 100 µg/ml denatured salmon sperm DNA at 42°C, and washed in 2X SSC and 0.5% SDS at 55°C for 15 minutes and encodes a protein having enzymatic funtion; and

D.) a nucleic acid sequence that is Genbank Accession No. X69822.

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8. (Amended) A vector[,] comprising the expression cassette of claim [1]2.

9. (Amended) The vector of claim 8[,] which is an *Agrobacterium* binary vector.

12. (Amended) A method of producing a transformed Poplar plant [with improved nitrogen metabolism] by transforming *in vitro* said plant with the expression cassette of claim 2.

16. (Amended) The method of claim [15] 12, wherein the plant is the hybrid *Populus tremula* X *P. alba*.

18. (Amended) The method of claim 12, wherein the said plant is transformed by infection with an [transformation step uses the] *Agrobacterium [tumifaciens] tumefaciens vector*

comprising a nucleic acid encoding glutamate synthetase.

20. (Amended) A transgenic plant produced by the method of claim [12] 18.

21. (Amended) An isolated reproductive unit from the transgenic plant of claim [15] 20, said unit comprising a nucleic acid encoding heterologous glutamine synthetase.

22. (Amended) A cell from the transgenic plant of claim [21]20, wherein said cell comprises a nucleic acid encoding heterologous glutamine synthetase .

29. (Amended) The transgenic plant of claim [28] 20, which is a hybrid of *Populus tremula* X *Populus alba*.

Appendix B

Abstract

Nitrogen is one of the principal factors limiting vegetative production. The present invention has improved the nitrogen metabolism in Poplar by integrating a transgene constitutively expressing a pine glutamine synthetase into the plant genome. The resulting transgenic trees exhibit higher growth rates, protein and chlorophyll contents, and leaf area than equivalent untransformed trees. It is contemplated that this approach to nitrogen improvement will be equally successful for all woody perennials. Provided with the invention is an expression cassette, a vector, and a method for increase glutamine synthetase activity in woody perennials, as well as transgenic woody perennials with enhanced nitrogen metabolism and accompanying phenotype.